CMLS, Cell. Mol. Life Sci. 60 (2003) 629–638
1420-682X/03/030629-10
© Birkhäuser Verlag, Basel, 2003

CMLS Cellular and Molecular Life Sciences

Research Article

CD4$^+$ T cell-mediated immunity against prion proteins

L. Stoltze [a,‡,*], H. Rezaei [b,c], G. Jung [d], J. Grosclaude [c], P. Debey [b], H. Schild [a] and H.-G. Rammensee [a]

[a] Institute for Cell Biology, Department of Immunology, University of Tübingen, Auf der Morgenstelle 15, 72076 Tübingen (Germany)
[b] Institut National de la Recherche Agronomique (INRA) Unité 806/EA2703, Muséum National d'Histoire Naturelle, 75005 Paris (France)
[c] INRA, Unité de Virologie et Immunologie Moléculaires, 78352 Jouy-en-Josas (France)
[d] Institute for Organic Chemistry, University of Tübingen, Auf der Morgenstelle 18, 72076 Tübingen (Germany)

Received 5 December 2002; received after revision 16 January 2003; accepted 5 February 2003

Abstract. The prion protein (PrP^C) is essential for susceptibility to transmissible spongiform encephalopathies. A specific conformer of this protein (PrP^{Sc}) is, according to the 'protein only' hypothesis, the principal or only component of the infectious agent, designated prion. Transmission of prions between species is often inefficient, resulting in low attack rates and/or prolonged incubation times and is ascribed to a 'species barrier' caused by differences in the amino acid sequence of PrP between recipient and donor. In this report, we demonstrate that these differences in amino acid sequence result in presentation of distinct peptides on major histocompatibility complex class II molecules. These peptides result in activation of specific CD4$^+$ T cells which leads to the induction of an effective immune response against foreign PrP as demonstrated by antibody production. Therefore, CD4$^+$ T cells represent a crucial component of the immune system to distinguish between foreign and self PrP.

Key words. T lymphocyte; prion protein; MHC class II.

Transmissible spongiform encephalopathies (TSEs), or prion diseases, are degenerative disorders of the central nervous system leading to motor dysfunction, dementia and death. Prion diseases include scrapie of sheep, bovine spongiform encephalopathy (BSE) in cattle and human diseases such as Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS) and fatal familial insomnia (FFI). More recently, variant CJD (vCJD), ascribed to consumption of BSE-contaminated products [1], has claimed over 100 victims. Neither humoral nor cellular immunological responses have been detected in prion diseases.

* Corresponding author.
‡ Present address: MRC Prion Unit, Institute of Neurology, University of London, Queen Square London, WC1N 3BG (United Kingdom), Fax + 44 20 7676 2180, e-mail: l.stoltze@prion.ucl.ac.uk

Transmissibility of scrapie was first demonstrated at the end of the 1930s [2]. The unusual resistance of the agent to UV irradiation suggested that it might be devoid of nucleic acid [3]. The 'protein only' hypothesis [4] in its updated version [5] proposes that the prion is a conformational isoform of the normal host protein PrP^C [6, 7] that is expressed in many tissues [8], albeit predominantly on the outer surface of neurons, attached by a glycosylphosphatidyl inositol (GPI) anchor. The abnormal conformer, when introduced into the organism, is thought to cause the conversion of PrP^C into a likeness of itself.
In prion disease, a largely protease-resistant, aggregated form of PrP, designated PrP^{Sc}, accumulates, mainly in brain. It is believed to be the principal or only constituent of the prion [5]. No differences in the primary structure of PrP^C and PrP^{Sc} have been detected, suggesting that they differ in their conformation [9]. While the tertiary structure of

PrP^{C} has been elucidated [10], that of PrP^{Sc} has not; however, the β sheet content of PrP^{Sc} was found to be high while that of PrP^{C} is low [11, 12]. The conclusion that some form of PrP is the essential, perhaps only constituent of the infectious agent is based on compelling biochemical and genetic evidence [13, 14]. The finding that PrP knockout (*Prnp$^{0/0}$*) mice were completely protected against scrapie disease and failed to propagate prions [15, 16] and that introduction of murine *Prnp* transgenes into these mice restored susceptibility to prions [17] is one of the main supports for the 'protein only' hypothesis.

Infection with prions from a different species results in prolonged incubation times, attributed to a 'species barrier' due to species-specific differences in the PrP amino acid sequence that hinder PrP^{C} to PrP^{Sc} conversion [18–20]. Transmission between certain species seemed to be impossible, as judged by clinical criteria. However, recent results suggest that at least in some such cases, prion replication and PrP^{Sc} accumulation do take place, albeit very late after inoculation and without clinical symptoms [21–23], thus representing a form of subclinical disease [24].

Characteristically, prion infections do not give rise to an obvious humoral or cellular immune response [15, 25, 26]. Following the discovery of PrP as an essential prerequisite for susceptibility to prion disease [27] and the observation that PrP is a cellular protein [7, 28], it has been widely accepted that no immune response is possible because of tolerance induced by the self protein. Indeed, immunization of PrP knockout mice with murine PrP^{C} gives rise to high titers of PrP antibodies. Paradoxically, the immune system seems to be involved in transport [29–31] and propagation [32] of infectivity.

Because foreign PrP can induce antibody responses in wild-type mice [33], we wondered whether the 'species barrier' might at least in part be due to an immune response elicited by the foreign PrP. We therefore investigated the CD4^{+} T cell response against foreign PrP molecules because CD4^{+} T cells are essential key players in mediating any efficient humoral and cellular immune response. In this report, we show for the first time that the immune system differentiates between host and foreign PrP^{C} with the assistance of CD4^{+} T cells. This observation suggests that not only humoral but also cellular immunity against foreign PrP molecules may occur.

Materials and methods

Mice and T cell lines

C57BL/6, BALB/c (Charles River, Sulzfeld, Germany), *Prnp$^{0/0}$* [34] and tg94 [35] mice (kindly provided by C. Weissmann, London, UK, and A. Aguzzi, Zürich, Switzerland) were kept under standard conditions. T cell lines 1VRQ and M222 were generated by immunization of *Prnp$^{0/0}$* mice with recombinant shPrP and DNA immunization with pAD-MD-CMV-PrP, respectively. All lines were cultured in αMEM (Sigma, Munich, Germany) containing 10% fetal calf serum (FCS) (PAN, Aidenbach, Germany), 50 units penicillin/streptomycin, 2 mM glutamine (c.c.pro, Neustadt, Germany), 50 µM 2-mercaptoethanol and restimulated weekly with irradiated C57BL/6 spleen cells.

Proteins, peptides and DNA

Recombinant shPrP (natural variant V136Q171) and mPrP (*PrnpA*) were expressed in *Escherichia coli* and purified as described for shPrP [36] and mPrP [37], respectively. Protein purity reached up to 100% as judged by silver staining and immunoblotting with antibody 6H4 (Prionics, Zürich, Switzerland). Yeast enolase was obtained from Sigma. Peptides were synthesized on a multiple-peptide synthesizer using Fmoc-amino acid and N-hydroxybenzotriazole/diisopropylcarbodiimide on polystyrene-1% divinyl benzene resin with trityl chloride linker. The purity was determined by HPLC equipped with mass and UV (214 nm) detectors. The plasmid pRSETA-mini-mPrP for expression of recombinant mPrP was a kind gift of K. Wüthrich, Zürich, Switzerland. Plasmid pAD-MD-CMV-PrP encoding the cDNA of mPrP (*PrnpA*) used for immunization was a kind gift of C. Weissmann, London, UK. All DNA sequences were confirmed by sequencing.

Immunizations

Mice 6–8 weeks old were immunized with 60 µg recombinant shPrP or mPrP in CFA (DIFCO, Detroit, Mich.) (1 : 1) in a total volume of 150 µl. Fifty microliters of each was injected subcutaneously (s.c.) in footpads and tail. DNA immunization was performed as described elsewhere [38]. In brief, 100 µl phosphate-buffered saline (PBS) containing 6.25 ng/µl cardiotoxin (Latoxan, Rosans, France) was injected intramuscularly (i.m.). Five days later, mice were immunized the same way with 100 µg plasmid DNA in PBS. All mice were sacrificed 10 days after immunization.

Thymidine incorporation assay

Draining lymph nodes from immunized mice were taken and 5 × 10^{5} cells were incubated in a 96-well plate with either protein (if amount not indicated 30 µg/ml), cells or concanavalin A (ConA) as indicated, or 1 µM peptide. For assays with T cell lines, 3 × 10^{4} T cells were plated with 5 × 10^{5} irradiated *Prnp$^{0/0}$* spleen cells and 90 nM peptide. All experiments were carried out in triplicate. After 24 h, 100 µl of the culture volume of 200 µl αMEM medium was taken for cytokine ELISA and 50 µl of fresh αMEM was added. After 44 h, 20 µl αMEM containing 1 µCi of ^{3}H (Amersham, Freiburg, Germany) were added and incorporated activity was harvested (INOTECH, Dottikon,

Germany) and measured at 72 h (1450 Microbeta Plus; WALLAC, Dreieich, Germany).

Flow cytometry

4×10^5 cells from T cell cultures were stained by either anti-CD8, anti-CD4 or the respective isotype control antibody. All antibodies were directly fluorescein isothiocyanate (FITC) labeled (BD Pharmingen, Heidelberg, Germany).

Immunoblot and ELISA

Immunoblot was performed by loading 400 ng of corresponding proteins on 12% SDS gels. After electrophoresis, protein was transferred by electroblot onto nitrocellulose membrane (Pharmacia, Freiburg, Germany). Bovine serum albumin-blocked membranes were then incubated for 45 min at room temperature with serum of mice 1:50 diluted in PBS. After washing, specific binding was detected by goat anti-mouse IgG coupled to HRP (1:5000, BD Pharmingen). Signals were detected using ECL kits (Pharmacia) and a photo image reader (Fuji, Stamford, Conn.).
Standard sandwich cytokine ELISA with 50 µl cell supernatant was performed using antibodies against the corresponding cytokine (BD Pharmingen). For ELISA with serum from immunized mice, the serum was diluted 1:500 and tested against 200 ng/ml of recombinant protein. Detection was performed using 1:3000 diluted goat anti-mouse IgG-HRP antibody (BD Pharmingen).

Results

PrP-specific T cells in *Prnp$^{0/0}$* and wild-type mice

To investigate whether PrP-specific T cells exist, *Prnp$^{0/0}$* mice [C57BL/6 × 129/Sv(ev) haplotype H-2^b] [34] were immunized s.c. with recombinant sheep PrP (shPrP), recombinant mouse PrP (mPrP) or i.m. with PrP cDNA. Since the *Prnp$^{0/0}$* mice had never been exposed to PrP^C, they were expected to develop a normal immune response against PrP. Indeed, all PrP^C species tested elicited T cells specific for the corresponding PrP molecule. Stimulation with irrelevant proteins such as enolase did not result in any T cell proliferation (fig. 1A) and cells from untreated mice did not respond at all (data not shown). More importantly, peptides of PrP molecules are naturally processed and presented by MHC molecules, as demonstrated by the fact that T cells from *Prnp$^{0/0}$* mice immunized with shPrP recognize spleen cells from wild-type C57BL/6 (haplotype H-2^b) and tg94 mice (*Prnp$^{0/0}$* mice transgenic for mPrP overexpressed in spleen) [39] but not *Prnp$^{0/0}$* mice (fig. 1C). This response is not due to an in vitro minor H response because of the mixed background of the *Prnp$^{0/0}$* mice, since cells of nonimmunized *Prnp$^{0/0}$* mice did not proliferate on stimulation with C57BL/6 or tg94 cells (data not shown). Cells from wild-type mice were slightly better recognized than cells from tg94 mice, although tg94 mice have a higher PrP expression in the spleen. This might be due to the chosen promoter of the transgene which limits expression to T and B cells [39]. Therefore, other cell populations such as dendritic cells, which are more potent in stimulating T cells, may lack PrP expression in these mice.
All immunizations of *Prnp$^{0/0}$* mice resulted in the generation of T cell lines which responded to C57BL/6 spleen cells. These established T cell lines were exclusively CD4$^+$, as demonstrated by flow cytometry analysis (fig. 2).
The experiments reported so far clearly demonstrated a specific immune response against PrP in *Prnp$^{0/0}$* mice and this led us to consider whether a similar response would be detectable in wild-type mice. After immunizing C57BL/6 mice with shPrP, we observed a similar T cell response when stimulating with protein (fig. 1B). However, these T cells responded only very weakly to wild-type or tg94 spleen cells after 3 days in culture (fig. 1D). In addition, T cells from *Prnp$^{0/0}$* mice responded similarly to mPrP and shPrP, whereas T cells from wild-type mice regularly responded up to five times better to shPrP than to mPrP (fig. 1E, F). Similar results were obtained by immunizing BALB/c mice (haplotype H-2^d), demonstrating that the observed effect is not limited to the H-2^b haplotype (data not shown). This difference most likely reflects the induction of tolerance against mPrP but not shPrP in wild-type mice and demonstrates the existence of species-specific T helper cell epitopes in PrP.

Interferon-γ and interleukin-2 production by PrP-specific T cells

To investigate which type of help T cells supply during an immune response against PrP and to demonstrate the T cell-specific proliferation, we determined the cytokines produced in the proliferation assays. T cells of *Prnp$^{0/0}$* origin showed interleukin-2 (IL-2) and interferon-γ (IFN-γ) production after stimulation with shPrP as well as after contact with wild-type or tg94 spleen cells, but not after exposure to irrelevant enolase (fig. 3A, E) or *Prnp$^{0/0}$* cells (fig. 3C, G). T cells from wild-type mice behaved similarly after protein stimulation (fig. 3B, F) and as expected from the thymidine incorporation assay produced only little IL-2 and IFN-γ on contact with tg94 and wild-type cells after 24 h in culture (fig. 3D, H). Under no circumstances were we able to detect IL-4 or IL-6 by cytokine ELISA. Consequently, the observed T cells represent a classical Th1 phenotype and should be able to provide help for B cells as well as for cytotoxic T cells (CTLs).

PrP-specific T cells recognize different MHC class II-presented peptides

To determine the specificity of PrP-reactive T cells in detail and to provide an explanation for the species-specific recognition of PrP molecules in wild-type mice, 21 shPrP

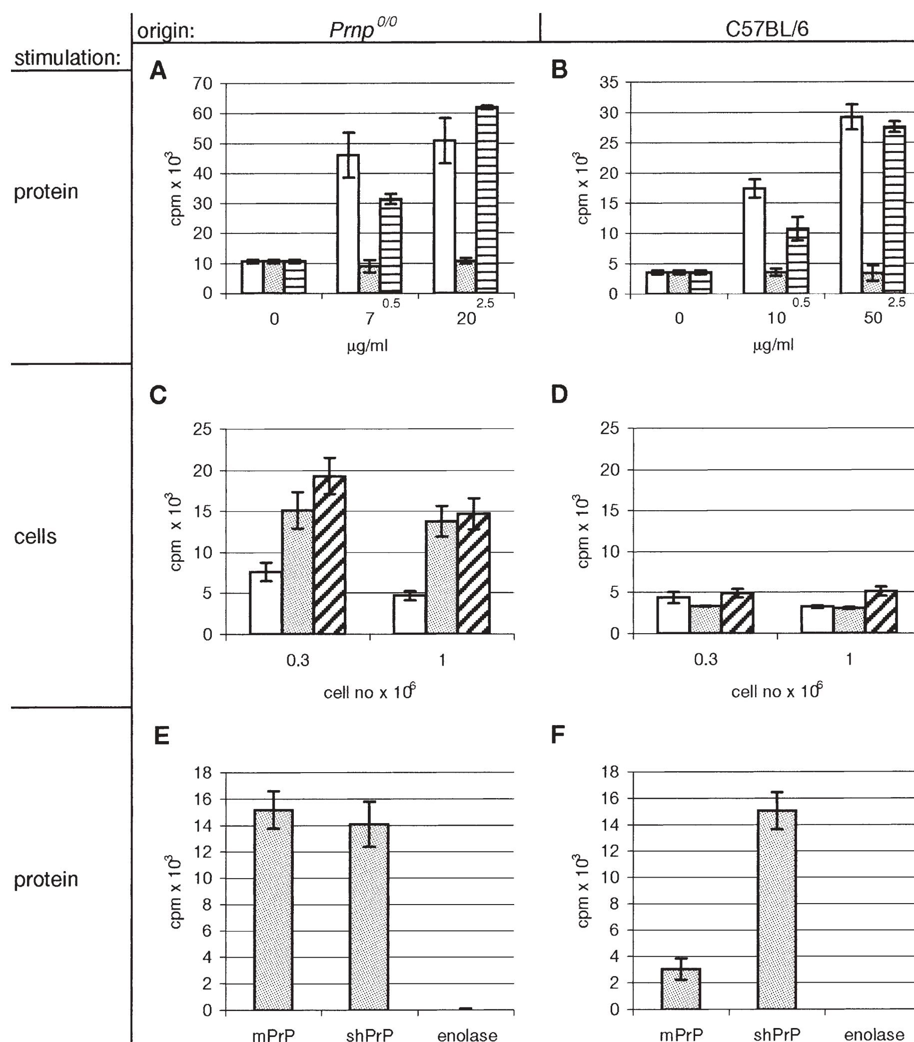

Figure 1. T cells against shPrP in *Prnp*$^{0/0}$ and C57BL/6 mice. Draining lymph nodes from immunized mice were taken into culture and T cell proliferation was measured after 72 h by a thymidine incorporation assay. Data from *Prnp*$^{0/0}$ (*A, C, E*) and C57BL/6 (*B, D, F*) mice are shown. Graphs *A–D* and *E*, *F* represent one experiment each including the standard deviation of triplicates. Stimulation with shPrP (open bars), enolase as irrelevant protein control (filled bars) and ConA (hatched bars) at the concentrations indicated (*A, B*); cells from *Prnp*$^{0/0}$ (open bars), tg94 (filled bars) or C57BL/6 (hatched bars) mice at the cell number indicated (*C, D*); the indicated proteins (*E, F*).

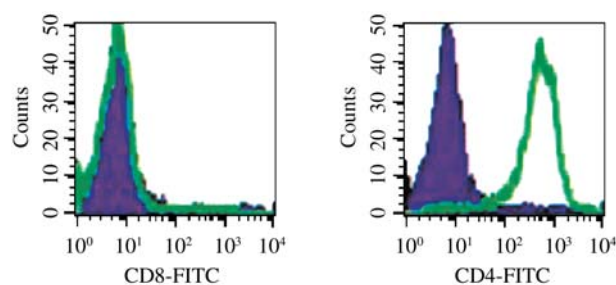

Figure 2. Characterization of T cells by flow cytometry. T cell cultures were tested by flow cytometry for the expression of CD8 or CD4 co-stimulatory molecules. Closed areas, FITC-labeled isotype control antibody; open areas, FITC-labeled anti-CD8 and anti-CD4 antibody.

peptides of 20 amino acids in length were synthesized. These peptides overlapped by 10 amino acids and covered the whole sequence of recombinant shPrP, excluding the N-terminal signal sequence and the C-terminal GPI linkage sequence (fig. 4). All peptides were then tested for recognition by different T cell lines or on ex vivo T cell cultures of immunized mice. T cell lines from *Prnp*$^{0/0}$ mice specific for shPrP reacted against peptides 8, 12, 14 and 16 (fig. 5A). However, upon further restimulation with wild-type C57BL/6 spleen cells, the reactivity against peptides 8 and 16 was lost. This could be due to the fact that peptides 8 and 16 are not generated from mPrP in cells or that they differ too much from the sheep homologue to induce cross-reactivity. Peptide 8 in particular reveals six differences in the amino acid sequence

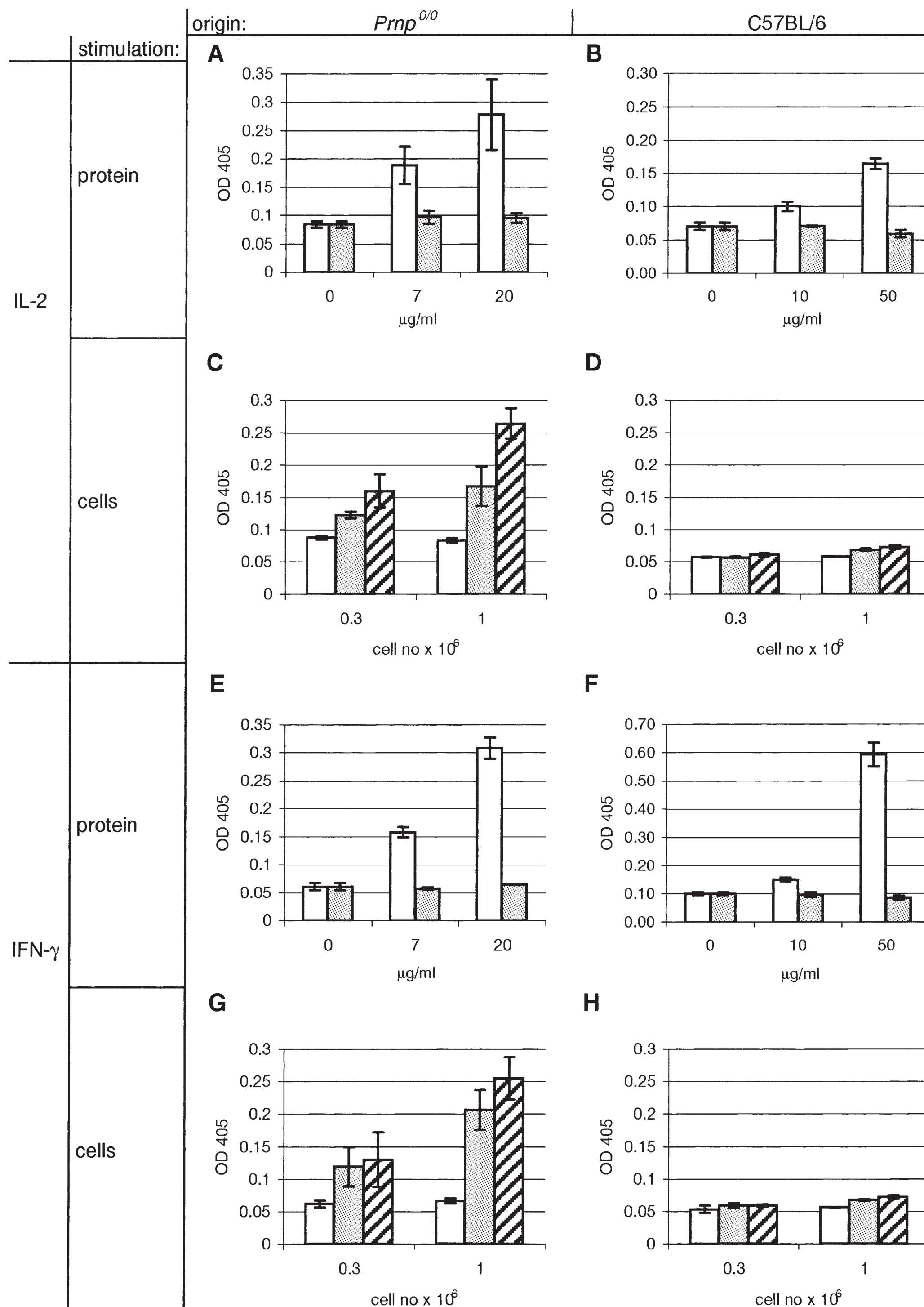

Figure 3. IFN-γ and IL-2 production by PrP-specific T cells. Draining lymph nodes from immunized mice were cultured and cytokine secretion in the supernatant was measured after 24 h by cytokine ELISA. Data from *Prnp*$^{0/0}$ (*A, C, E, G*) and C57BL/6 (*B, D, F, H*) showing IL-2 (*A–D*) and IFN-γ (*E–H*) secretion. Graphs represent one experiment including the standard deviation of triplicates. Stimulation with shPrP (open bars) and enolase as irrelevant protein control (filled bars) at the concentration indicated (*A, B, E, F*); cells from *Prnp*$^{0/0}$ (open bars), tg94 (filled bars) or C57BL/6 (hatched bars) mice at the cell number indicated (*C, D, G, H*).

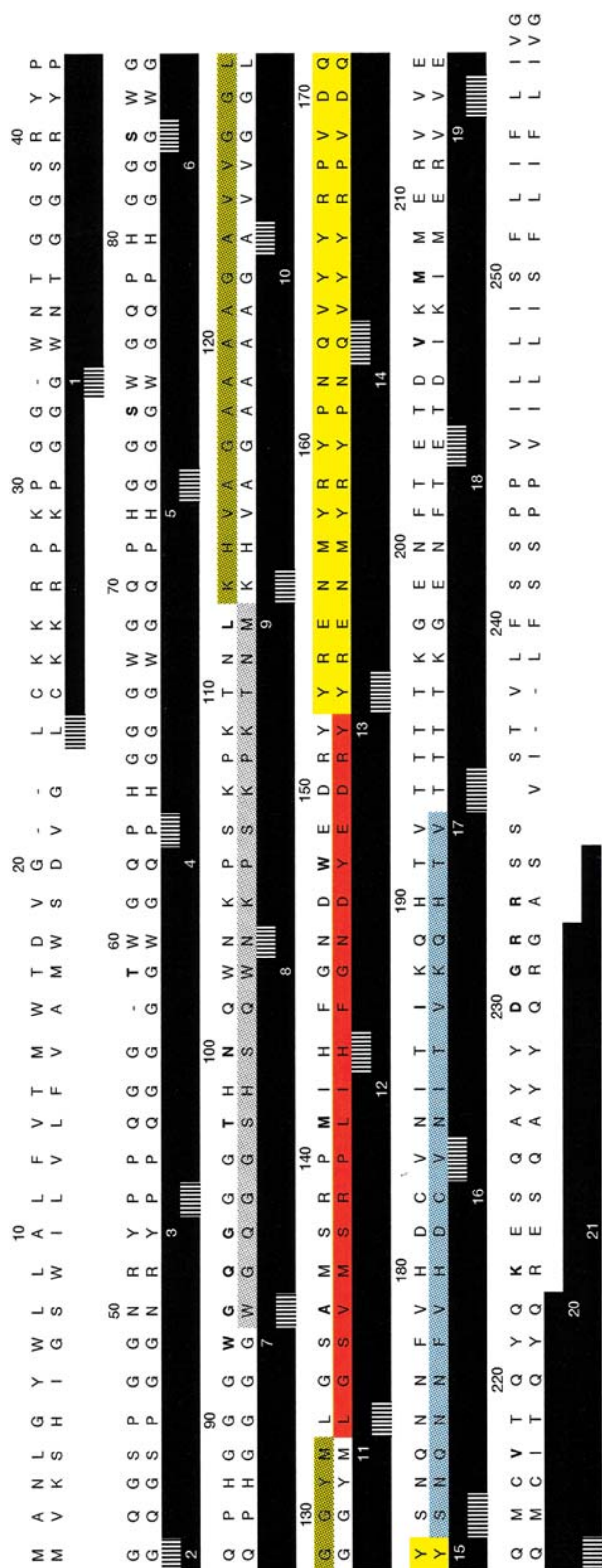

Figure 4. H2-A^b epitopes in shPrP and mPrP. Comparison of shPrP and mPrP amino acid sequences. Upper lane mPrP, lower lane shPrP. Both sequences are aligned and numbered according to shPrP. Numbers of overlapping peptides used for epitope mapping are given in black bars which represent the peptides, each starting with a hatched area. Sequence differences are in bold letters and epitopes described in the text are marked with different-colored bars.

(fig. 4), which could certainly result in loss of anchor positions required for binding to the MHC class II molecule [40]. On the other hand, T cell lines from *Prnp*$^{0/0}$ mice specific for mPrP reacted only against peptides 10 and 14 (fig. 5B), which already demonstrate differences in the peptide repertoire of MHC class II-presented peptides from PrP of different species.

The ex vivo stimulation with peptides revealed that shPrP-specific T cells from *Prnp*$^{0/0}$ mice reacted against peptides 12 and 14 as well as peptides 8 and 10 (fig. 5C). T cells from wild-type mice specific for shPrP reacted against peptide 12 and less frequently against peptide 8 (fig. 5E and data not shown), suggesting tolerance against peptide 14, which is identical in mPrP and shPrP. The dominant peptide for mPrP-specific T cells of *Prnp*$^{0/0}$ origin was peptide 14 and to a lesser extent peptide 10 (fig. 5D), thus correlating well with the data obtained from the corresponding T cell lines (fig. 5B). Not surprisingly, we were unable to raise T cells in wild-type mice against mPrP (fig. 5F) because of the apparent tolerance against all MHC class II-presented mPrP peptides. The lower proliferation of T cells against exogenously added peptides, even though similar molar amounts of peptide and protein were used, can have several causes. First of all the response against the whole protein represents a response against all available MHC class II epitopes, whereas the peptides represent only one epitope each. Furthermore, the addition of exogenous peptide may result in less efficient MHC peptide loading because proteases may digest free peptides and peptide exchange may be less efficient on the cell surface. Finally, the active 20mer peptide may contain the relevant MHC epitope but not represent the optimal epitope which may differ slightly in size.

Antibody responses against shPrP

In all mice immunized with shPrP we were able to detect a specific antibody response by immunoblot and ELISA, demonstrating the in vivo relevance of the previously observed CD4+ T cell response. As expected, antibodies from *Prnp*$^{0/0}$ mice revealed no species specificity. In C57BL/6 mice, however, we detected antibodies against shPrP cross-reacting in immunoblot assays against mPrP. This specificity was only weakly detectable in serum from BALB/c mice (fig. 6A). The observed signals were not artifacts, because serum of untreated mice as well as the secondary antibody alone showed no signal, even after overexposure (fig. 6A). Cross-reactivity detected in immunoblot experiments seemed to be due to similar linear peptide epitopes, because ELISA experiments against recombinant protein revealed no cross-reactivity, except for serum from *Prnp*$^{0/0}$ mice (fig. 6B). Peptide ELISA revealed that all antibodies reacted mainly against peptide 3 (data not shown). In serum from *Prnp*$^{0/0}$ and C57BL/6 mice, reactivity against peptide 1 was also detected,

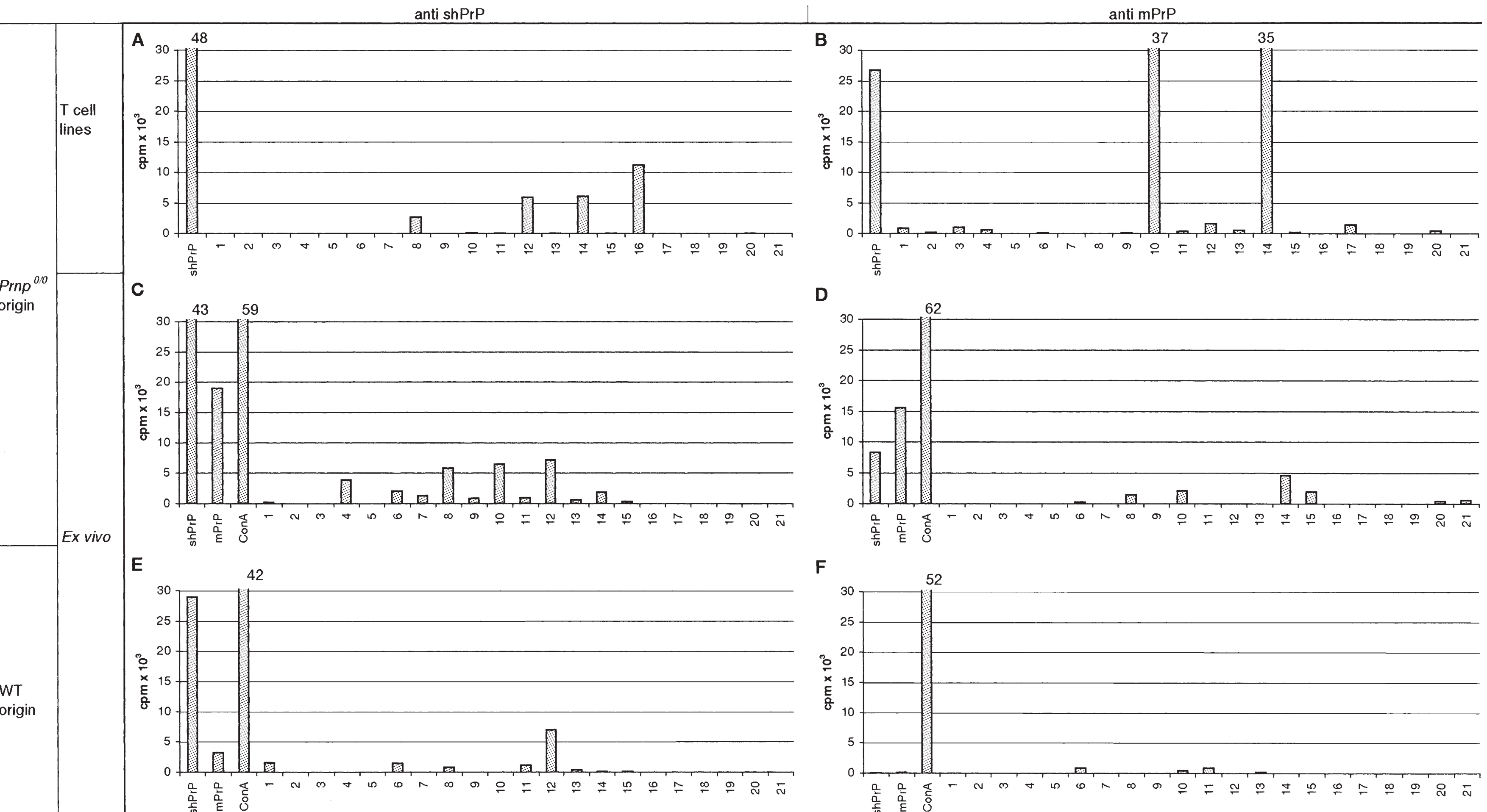

Figure 5. Peptides recognized by PrP-specific T cells. Thymidine incorporation assays with peptides covering the shPrP sequence were performed on T cell lines and ex vivo T cell cultures. Background was subtracted and the y-axis standardized to 30 kcpm; higher values are indicated. Graphs *A, B* and *C–F* represent one experiment each. Shown are the T cell line 1VRQ which originates from shPrP-immunized *Prnp*$^{0/0}$ mice (background: 0.9 kcpm) (*A*); the T cell line M222 which originates from mPrP cDNA immunized *Prnp*$^{0/0}$ mice (background: 14.9 kcpm) (*B*); ex vivo T cell culture of *Prnp*$^{0/0}$ mice immunized with shPrP (background: 10.4 kcpm) (*C*) or mPrP (background: 5.7 kcpm) (*D*) and C57BL/6 mice immunized with shPrP (background: 8.7 kcpm) (*E*) or mPrP (background: 5.5 kcpm) (*F*).

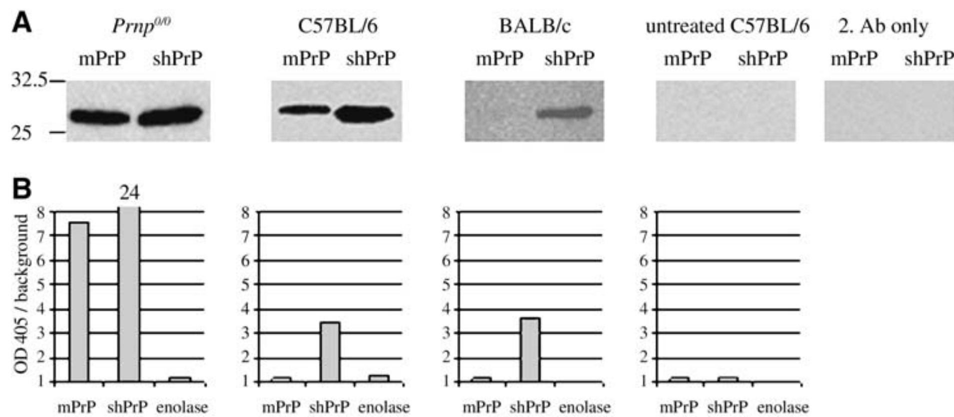

Figure 6. Characterization of antibody responses against shPrP. (*A*) Immunoblots with serum from mice immunized with shPrP. The serum from indicated mice was tested on recombinant mPrP and shPrP. As controls, blots with serum of an untreated C57BL/6 mouse and the secondary antibody only are shown. Blots for untreated *Prnp*$^{0/0}$ and BALB/c appeared similar. Molecular weight markers are indicated. (*B*) Corresponding ELISA with serum from the same mice. Signals against indicated proteins are shown. For easier comparison, obtained values were divided by the background and y-axis standardized; higher values are indicated.

which might explain the difference in cross-reactivity detected in the immunoblot of C57BL/6 and BALB/c (fig. 6A). Minor reactivity for all antibodies was detectable against peptides 14 and 15 (data not shown).

Discussion

We investigated the CD4$^+$ T cell-mediated immune response against PrP^C from different species and were able to demonstrate the existence of shPrP-specific CD4$^+$ T cells in *Prnp*$^{0/0}$, C57BL/6 and BALB/c mice (fig. 1 and data not shown), thereby showing the existence of MHC class II epitopes in the H-2^b and H-2^d haplotype. Similarly, we demonstrated the existence of mPrP-specific CD4$^+$ T cells in *Prnp*$^{0/0}$ mice (fig. 1). Using T cell lines established from *Prnp*$^{0/0}$ mice as well as ex vivo T cell cultures of immunized mice that had undergone peptide stimulation, we were able to reveal peptides containing the H2-A^b epitopes of shPrP and mPrP (H-2A^b is the only MHC class II molecule of C57BL/6 [41]). For shPrP, the dominant epitopes were peptides 12 (shPrP sequence 133–152) and 14 (shPrP 153–172). The dominant H-2A^b epitopes of mPrP consisted of peptides 10 (shPrP 113–132) and 14. Nevertheless, peptide 12 can be expected to also contain at least a subdominant mPrP H-2A^b epitope because the T cell line 1VRQ, of shPrP-immunized *Prnp*$^{0/0}$ origin, was stimulated with C57BL/6 cells (which are able to bind the equivalent mouse peptide [42]) and finally recognized the corresponding sheep peptide. The reason why it has a subdominant status and is not recognized by T cells after immunization of *Prnp*$^{0/0}$ mice with mPrP might be a case of immunodominance [43, 44]. However, the crucial observation is that CD4$^+$ T cells of C57BL/6 mice differentiate between sheep and mouse PrP^C by the virtue of peptide 12 (fig. 5E). Additionally, T cells from shPrP-immunized BALB/c mice recognize shPrP much better than mPrP (data not shown), which implies that the observed effect is not limited to one haplotype but has general significance. This observation of differential presentation of peptides on MHC class II molecules is in line with reports describing that a difference of one amino acid in a peptide sequence is sufficient to result in differential antigen processing and presentation on MHC molecules [43, 45, 46].

To demonstrate that the observed T cell response has a functional in vivo relevance, we investigated whether T cell-dependent B cell responses had occurred in the immunized mice. In our model system, all corresponding B cell responses observed reflect the picture seen for T cells. In wild-type mice, antibodies were capable of differentiating between native shPrP and mPrP, which was not the case in *Prnp*$^{0/0}$ mice (fig. 6B). The observed cross-reactivity in immunoblot assays was probably due to similar peptide epitopes (fig. 6A and data not shown). These data are in line with previous observations [33, 47–50].

The fact that we were able to observe a functional CD4$^+$ T cell response against foreign PrP^C molecules in wild-type mice leads us to reconsider some issues of prion biology from an immunological point of view. The relevance of our observation in terms of prion infection could be questioned given that we investigated the T cell response against PrP^C but not PrP^{Sc}. However, this objection is not valid. In a natural situation, infection occurs between different species and the material containing infectivity never consists of pure PrP^{Sc}, but also contains PrP^C. Consequently, assuming that an immune response against PrP^C will also effect PrP^{Sc} seems reasonable, because so far no convincing evidence exists that antibodies or any

other components of the immune system are able to differentiate between PrP^{C} and PrP^{Sc}. Thus the immune system sees a foreign protein against which it is able to develop a $CD4^{+}$ T cell response and, as shown, an antibody-mediated immune response. In addition, that the observed $CD4^{+}$ T cell response might lead to cellular immunity by CTLs or that other mechanisms of the immune system are activated cannot be excluded. Taking into account all the recently published data which demonstrate that various effector mechanisms of the innate and humoral immune response either in vitro [51, 52] or in vivo [53–55] have an effect on prion propagation and incubation time of disease, it seems reasonable to assume that activation of the immune system by the presence of foreign PrP^{C} will influence the incubation time of disease. A role for $CD4^{+}$ T cells in these effector mechanisms is supported by the interesting observation that vCJD patients have a reduced frequency of a certain MHC class II allele [56]. These patients could thus constitute a group of individuals with immune systems unable to mount certain effector mechanisms that are essential to extend the incubation time of disease beyond their natural life span.

This scenaric stimulates speculation as to whether the phenomenon of the species barrier in prion diseases relies only on the current discussed mechanism of less efficient conformational transfer from foreign PrP^{Sc} to the host PrP^{C} molecule due to differences in the amino acid sequence [18–20] or whether components of the immune system are involved as well. An important question developing out of this assumption is whether the permanent uptake of foreign PrP^{C} by food products results in oral tolerance [57] or protective immunity.

Summarizing our observation concerning the $CD4^{+}$ T cell response and the recently published data of effector mechanisms influencing incubation times of prion disease, the immune system seems not only to play a role in transport and replication of infectivity but is also able to have a protective function under certain circumstances. Unquestionably, information obtained from differential MHC class II epitopes in PrP from different species will be a very useful tool in developing pre- or post-exposure immunization protocols which might have a positive effect on various stages of neurodegenerative prion diseases.

Acknowledgements. The authors wish to thank L. Yakes, M. Hofmann and C. Weissmann for critical reading of the manuscript and P. Decker, S. Tenzer and N. Setzler for excellent technical advice. This work was supported by grants from the Deutsche Forschungsgemeinschaft [Leibnizprogram H.G.R. (Ra369/4-1], Sonderforschungsbereich 510 to H. S. and G. J. and the European Union (Biomed 95-0263).

1 Will R. G., Ironside J. W., Zeidler M., Cousens S. N., Estibeiro K., Alperovitch A. et al. (1996) A new variant of Creutzfeldt-Jakob disease in the UK. Lancet **347:** 921–925
2 Cuille, J. et al. (1938) La tremblante du mouton est bien inoculable. C. R. Acad. Sci. Paris **206:** 78–79
3 Alper T., Cramp W. A., Haig D. A. and Clarke M. C. (1967) Does the agent of scrapie replicate without nucleic acid? Nature **214:** 764–766
4 Griffith J. S. (1967) Self-replication and scrapie. Nature **215:** 1043–1044
5 Prusiner S. B. (1989) Scrapie prions. Annu. Rev. Microbiol. **43:** 345–374
6 Basler K., Oesch B., Scott M., Westaway D., Walchli M., Groth D. F. et al. (1986) Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. Cell **46:** 417–428
7 Oesch B., Westaway D., Walchli M., McKinley M. P., Kent S. B., Aebersold R. et al. (1985) A cellular gene encodes scrapie PrP 27–30 protein. Cell **40:** 735–746
8 Bendheim P. E., Brown H. R., Rudelli R. D., Scala L. J., Goller N. L., Wen G. Y. et al. (1992) Nearly ubiquitous tissue distribution of the scrapie agent precursor protein. Neurology **42:** 149–156
9 Stahl N., Baldwin M. A., Teplow D. B., Hood L., Gibson B. W., Burlingame A. L. et al. (1993) Structural studies of the scrapie prion protein using mass spectrometry and amino acid sequencing. Biochemistry **32:** 1991–2002
10 Riek R., Hornemann S., Wider G., Glockshuber R. and Wüthrich K. (1997) NMR characterization of the full-length recombinant murine prion protein, mPrP(23–231). FEBS Lett. **413:** 282–288
11 Caughey B. W., Dong A., Bhat K. S., Ernst D., Hayes S. F. and Caughey W. S. (1991) Secondary structure analysis of the scrapie-associated protein PrP 27–30 in water by infrared spectroscopy. Biochemistry **30:** 7672–7680
12 Pan K. M., Baldwin M., Nguyen J., Gasset M., Serban A., Groth D. et al. (1993) Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. Proc. Natl. Acad. Sci. USA **90:** 10962–10966
13 Weissmann C. (1999) Molecular genetics of transmissible spongiform encephalopathies. J. Biol. Chem. **274:** 3–6
14 Prusiner S. B. (1998) The prion diseases. Brain Pathol. **8:** 499–513
15 Büler H., Aguzzi A., Sailer A., Greiner R. A., Autenried P., Aguet M. et al. (1993) Mice devoid of PrP are resistant to scrapie. Cell **73:** 1339–1347
16 Sailer A., Büler H., Fischer M., Aguzzi A. and Weissmann C. (1994) No propagation of prions in mice devoid of PrP. Cell **77:** 967–968
17 Fischer M., Rulicke T., Raeber A., Sailer A., Moser M., Oesch B. et al. (1996) Prion protein (PrP) with amino-proximal deletions restoring susceptibility of PrP knockout mice to scrapie. EMBO J. **15:** 1255–1264
18 Scott M., Foster D., Mirenda C., Serban D., Coufal F., Walchli M. et al. (1989) Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques. Cell **59:** 847–857
19 Priola S. A. and Chesebro B. (1995) A single hamster PrP amino acid blocks conversion to protease-resistant PrP in scrapie-infected mouse neuroblastoma cells. J. Virol. **69:** 7754–7758
20 Wopfner F., Weidenhofer G., Schneider R., Brunn A. von, Gilch S., Schwarz T. F. et al. (1999) Analysis of 27 mammalian and 9 avian PrPs reveals high conservation of flexible regions of the prion protein. J. Mol. Biol. **289:** 1163–1178
21 Race R. and Chesebro B. (1998) Scrapie infectivity found in resistant species. Nature **392:** 770
22 Hill A. F., Joiner S., Linehan J., Desbruslais M., Lantos P. L. and Collinge J. (2000) Species-barrier-independent prion replication in apparently resistant species. Proc. Natl. Acad. Sci. USA **97:** 10248–10253
23 Race R., Raines A., Raymond G. J., Caughey B. and Chesebro B. (2001) Long-term subclinical carrier state precedes scrapie

replication and adaptation in a resistant species: analogies to bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease in humans. J. Virol. **75:** 10106–10112
24 Thackray A. M., Klein M. A., Aguzzi A. and Bujdoso R. (2002) Chronic subclinical prion disease induced by low-dose inoculum. J. Virol. **76:** 2510–2517
25 Kingsbury D. T., Smeltzer D. A., Gibbs C. J. and Gajdusek D. C. (1981) Evidence for normal cell-mediated immunity in scrapie-infected mice. Infect. Immun. **32:** 1176–1180
26 Porter D. D., Porter H. G. and Cox N. A. (1973) Failure to demonstrate a humoral immune response to scrapie infection in mice. J. Immunol. **111:** 1407–1410
27 Bolton D. C., McKinley M. P. and Prusiner S. B. (1982) Identification of a protein that purifies with the scrapie prion. Science **218:** 1309–1311
28 Chesebro B., Race R., Wehrly K., Nishio J., Bloom M., Lechner D. et al. (1985) Identification of scrapie prion protein-specific mRNA in scrapie-infected and uninfected brain. Nature **315:** 331–333
29 Klein M. A., Frigg R., Flechsig E., Raeber A. J., Kalinke U., Bluethmann H. et al. (1997) A crucial role for B cells in neuroinvasive scrapie. Nature **390:** 687–690
30 Aucouturier P., Geissmann F., Damotte D., Saborio G. P., Meeker H. C., Kascsak R. et al. (2001) Infected splenic dendritic cells are sufficient for prion transmission to the CNS in mouse scrapie. J. Clin. Invest **108:** 703–708
31 Huang F. P., Farquhar C. F., Mabbott N. A., Bruce M. E. and MacPherson G. G. (2002) Migrating intestinal dendritic cells transport PrP(Sc) from the gut. J. Gen. Virol. **83:** 267–271
32 Brown K. L., Stewart K., Ritchie D. L., Mabbott N. A., Williams A., Fraser H. et al. (1999) Scrapie replication in lymphoid tissues depends on prion protein-expressing follicular dendritic cells. Nat. Med. **5:** 1308–1312
33 Kascsak R. J., Rubenstein R., Merz P. A., Tonna-DeMasi M., Fersko R., Carp R. I. et al. (1987) Mouse polyclonal and monoclonal antibody to scrapie-associated fibril proteins. J. Virol. **61:** 3688–3693
34 Büeler H., Fischer M., Lang Y., Bluethmann H., Lipp H. P., DeArmond S. J. et al. (1992) Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. Nature **356:** 577–582
35 Brandner S., Raeber A., Sailer A., Blattler T., Fischer M., Weissmann C. et al. (1996) Normal host prion protein (PrPC) is required for scrapie spread within the central nervous system. Proc. Natl. Acad. Sci. USA **93:** 13148–13151
36 Rezaei H., Marc D., Choiset Y., Takahashi M., Hui Bon H. G., Haertle T. et al. (2000) High yield purification and physicochemical properties of full-length recombinant allelic variants of sheep prion protein linked to scrapie susceptibility. Eur. J. Biochem. **267:** 2833–2839
37 Zahn R., Schroetter C. von and Wuthrich K. (1997) Human prion proteins expressed in *Escherichia coli* and purified by high-affinity column refolding. FEBS Lett. **417:** 400–404
38 Stoltze L., Dick T. P., Deeg M., Pommerl B., Rammensee H. G. and Schild H. (1998) Generation of the vesicular stomatitis virus nucleoprotein cytotoxic T lymphocyte epitope requires proteasome-dependent and -independent proteolytic activities. Eur. J. Immunol. **28:** 4029–4036
39 Raeber A. J., Sailer A., Hegyi I., Klein M. A., Rulicke T., Fischer M. et al. (1999) Ectopic expression of prion protein (PrP) in T lymphocytes or hepatocytes of PrP knockout mice is insufficient to sustain prion replication. Proc. Natl. Acad. Sci. USA **96:** 3987–3992
40 Rötzschke O. and Falk K. (1994) Origin, structure and motifs of naturally processed MHC class II ligands. Curr. Opin. Immunol. **6:** 45–51
41 Mathis D. J., Benoist C., Williams V. E., Kanter M. and McDevitt H. O. (1983) Several mechanisms can account for defective E alpha gene expression in different mouse haplotypes. Proc. Natl. Acad. Sci. USA **80:** 273–277
42 Souan L., Tal Y., Felling Y., Cohen I. R., Taraboulos A. and Mor F. (2001) Modulation of proteinase-K resistant prion protein by prion peptide immunization. Eur. J. Immunol. **31:** 2338–2346
43 Sercarz E. E., Lehmann P. V., Ametani A., Benichou G., Miller A. and Moudgil K. (1993) Dominance and crypticity of T cell antigenic determinants. Annu. Rev. Immunol. **11:** 729–766
44 Latek R. R. and Unanue E. R. (1999) Mechanisms and consequences of peptide selection by the I-Ak class II molecule. Immunol. Rev. **172:** 209–228
45 Antoniou A. N., Blackwood S. L., Mazzeo D. and Watts C. (2000) Control of antigen presentation by a single protease cleavage site. Immunity **12:** 391–398
46 Nelson C. A., Petzold S. J. and Unanue E. R. (1994) Peptides determine the lifespan of MHC class II molecules in the antigen-presenting cell. Nature **371:** 250–252
47 Groschup M. H. and Pfaff E. (1993) Studies on a species-specific epitope in murine, ovine and bovine prion protein. J. Gen. Virol. **74:** 1451–1456
48 Prusiner S. B., Groth D., Serban A., Koehler R., Foster D., Torchia M. et al. (1993) Ablation of the prion protein (PrP) gene in mice prevents scrapie and facilitates production of anti-PrP antibodies. Proc. Natl. Acad. Sci. USA **90:** 10608–10612
49 Krasemann S., Groschup M. H., Harmeyer S., Hunsmann G. and Bodemer W. (1996) Generation of monoclonal antibodies against human prion proteins in PrP0/0 mice. Mol. Med. **2:** 725–734
50 Zanusso G., Liu D., Ferrari S., Hegyi I., Yin X., Aguzzi A. et al. (1998) Prion protein expression in different species: analysis with a panel of new mAbs. Proc. Natl. Acad. Sci. USA **95:** 8812–8816
51 Peretz D., Williamson R. A., Kaneko K., Vergara J., Leclerc E., Schmitt-Ulms G. et al. (2001) Antibodies inhibit prion propagation and clear cell cultures of prion infectivity. Nature **412:** 739–743
52 Enari M., Flechsig E. and Weissmann C. (2001) Scrapie prion protein accumulation by scrapie-infected neuroblastoma cells abrogated by exposure to a prion protein antibody. Proc. Natl. Acad. Sci. USA **98:** 9295–9299
53 Heppner F. L., Musahl C., Arrighi I., Klein M. A., Rulicke T., Oesch B. et al. (2001) Prevention of scrapie pathogenesis by transgenic expression of anti-prion protein antibodies. Science **294:** 178–182
54 Sigurdsson E. M., Brown D. R., Daniels M., Kascsak R. J., Kascsak R., Carp R. et al. (2002) Immunization delays the onset of prion disease in mice. Am. J. Pathol. **161:** 13–17
55 Sethi S., Lipford G., Wagner H. and Kretzschmar H. (2002) Postexposure prophylaxis against prion disease with a stimulator of innate immunity. Lancet **360:** 229–230
56 Jackson G. S., Beck J. A., Navarrete C., Brown J., Sutton P. M., Contreras M. et al. (2001) HLA-DQ7 antigen and resistance to variant CJD. Nature **414:** 269–270
57 Nagler-Anderson C. (2001) Man the barrier! Strategic defences in the intestinal mucosa. Nat. Rev. Immunol. **1:** 59–67